

**United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Pesticide Data Program**

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Title: Determination of LOD and LOQ for Chromatographic Methods		
Revision: Original	Replaces:	Effective: 02/01/96

1. Purpose:

To provide standard operating procedures for determining the Limit of Detection (LOD) and Limit of Quantitation (LOQ) for analytes reported to the USDA/AMS Pesticide Data Program.

2. Scope:

This SOP shall be followed by all analytical laboratories which are conducting residue studies for PDP. This includes laboratories conducting stability and other studies which may impact the program.

3. Definitions:

Refer to section 6.7 (definitions).

4. Outline of Procedure:

- Determination of Method Noise
- Three Scenarios Occurring in Chromatographic Systems
- Estimation of LOD
- Estimation of LOQ
- Determination/Verification of LOD/LOQ
- LOD Checks
- Definitions

5. References:

Chemist Qualification document from Robert Epstein and summarized by Terry Jackson
with State participant comments, 4/23/92
Validation of Methods Used in the Florida Department of Agriculture and Consumer

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Services' Chemical Residue Laboratory, Parker, G.A., JAOAC, 74, No. 5, pp. 868-871, 1991

GLP Meeting with EPA/OPP, EPA/OCM, USDA/AMS, and USDA/AMS GLP Committee, 4/28/92

GLP Meeting with USDA/AMS GLP Committee and Robert Epstein, 4/29/92

Evaluation of Analytical Methods Used for Regulation of Foods and Drugs, Horwitz, W., Analytical Chemistry, Vol. 54, No. 1, pp. 67A-76A., 1982

Quality Assurance Principles for Analytical Laboratories, Garfield, F., AOAC, 1991

Quality Assurance of Chemical Measurements, Taylor, J.T., Lewis Publishers, 1989

Letter, Martha Lamont to PDP participants, May 5, 1992

Quality Assurance Officer's Meeting, February 21-23, 1995

Quality Assurance Committee Conversations, March 1995-Jan 1996

FDACS QA/QC Guideline Document, Section 14

PDP QC-10 Drafts, May, 1995-January, 1996

6. Specific Procedures:

6.1 Determination of Method Noise

- a. The Method Noise Determination process must be completed for all required PDP analytes.
 - b. Method noise will be determined utilizing instruments and operating conditions which are routinely used for the analysis of samples. Noise for the LOD and LOQ calculations will be determined by examining Chromatograms of the blank commodity in the Chromatographic Time Segment (CTS) of the pesticides of interest.
 - c. Method noise is defined as a combination of: drift noise, high frequency noise, low frequency noise, matrix noise, peak to peak noise and/or distinct chromatographic peaks, which occur in the CTS, which would interfere with reliable identification of the analyte. The CTS will be chosen based on the reproducibility of retention times and the variability of the baseline of the analytical
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system being evaluated.

- d. In order to establish LODs and LOQs which are reliably obtained in the day-to-day operation of the laboratory, commodities with significant matrix backgrounds will be chosen for the method noise calculation.

6.2 Three Scenarios Occurring in Chromatographic Systems

Note: Combinations of the three approaches can be used on a single instrument/column/detection system at different CTS (See definitions section 6.7).

6.2.1 Essentially Flat Baseline (Peak to Peak Noise Determination)

- a. Peak-to-peak noise determinations will be made when the blank commodity shows no distinct peaks in the CTS, producing an essentially flat baseline.
- b. Noise measurements for CTSs of the chromatographic systems will be made on at least three blank matrices.
- c. The peak-to-peak noise value for a certain CTS will be assigned to all analytes of interest, which elute in that CTS.
- d. The average calculated peak-to-peak noise in height, area or millivolts in the CTS of interest will be used in the equation to calculate LOD.

6.2.2 Small Interfering Peaks (Distinct Chromatographic Peak Determination)

- a. Distinct chromatographic peak determinations will be made when the blank commodity consistently shows distinct peaks (which are not resolvable) in CTS of interest.
 - b. Distinct peak measurements for CTSs of the chromatographic systems will be made on at least three matrices.
 - c. The distinct peak measurement in the CTS will be calculated in height, area
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or millivolts.

- d. Define in your internal SOP how the distinct peak measurement is used in the calculation of LOD (ie., worst case peak measurement or statistically based measurement).

6.2.3 Large Interfering Peaks (Unable to Detect)

- a. When the blank commodity shows matrix peaks which are greater than one-half full scale, when examined visually under the routine operating conditions, these peaks will not be included in noise determinations.
- b. In these cases, the peak will be designated a matrix interference and the analyte will be reported as "Unable to Detect".
- c. In some cases large matrix peaks obscure broad areas of the chromatogram. In those CTSs none of the eluting analytes for that commodity/CTS can be included in the screening list.

6.3 Estimation of LOD

- a. Estimation of LOD will be done on all required, single analysis or new PDP analytes. LOD will be estimated in ppm by substituting the calculated method noise level multiplied by approximately three into the ppm calculation equation using the calibration procedure for routine sample analysis.
- b. The reported LOD will be the highest value obtained using the primary identification technique and the confirmation technique (see section 6.7).

6.4 Estimation of LOQ

- a. Estimation of LOQ will be done on all required, single analysis or new
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PDP analytes. LOQ will be estimated in ppm by substituting the calculated method noise level multiplied by approximately ten into the ppm calculation equation using the calibration procedure for routine sample analysis.

- b. The reported LOQ will be the highest value obtained using the primary identification technique and the confirmation technique.

6.5 Determination/Verification of LOD

- a. All calculated LOD (see section 6.3.a) must be verified by fortifying duplicate blank commodities at approximately the LOD level and subjecting them to the analytical method.
- b. Verification consists of the observation of detectable peaks in the chromatogram at three times the current method noise level (run within the last three months). Variability is expected to be high. Therefore, recoveries can be reported as present or not present. If detectable peaks are not observed, the LOD must be reestimated and the verification repeated.

6.6 LOD Checks

- a. The LOD should be checked, at least yearly, by the injection of a dilution (keeping matrix effect equivalent to that of a LOD spike) of the fortified samples which accompany each set.
 - b. The LOD should be checked when analytical/instrumental changes result in lowered sensitivity.
 - c. LODs may be raised for analytes in an individual sample set at the discretion of Technical Program Manager.
 - d. LODs may not be lowered without verification subject to the analytical method, Technical Program Manager approval and QA review.
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6.7 Definitions:

Chromatographic Time Segment (CTS) - The segment along the baseline of a chromatogram used in the determination of Method Noise (eg., a broad CTS- the length of the entire chromatogram, a narrow CTS- an elution window of one or more analytes).

Blank Matrix - a matrix that does not produce an analytical response by the analytical method under investigation for the analyte(s) of interest.

Distinct Chromatographic Peak - A peak that displays an essentially gaussian shape and is a least 5 times the peak height of the matrix plus high frequency noise.

LOD - Limit of Detection, approximately 3 times the average of peak to peak noise value or approximately 3 times the largest distinctive peak in the retention window for the analyte of interest.

LOQ - Limit of Quantitation, approximately 10 times method noise (or approximately 3 times LOD).

Worst Case Matrix - The matrix that produces the highest average noise for a specified commodity group.

Drift Noise - Drift appears as a continuous increase or decrease of signal in the chromatogram. This source of noise is typically due to fluctuation in variables such as temperature, pressure, and flow as well as electronic and electrical variations. Excess drift makes it impossible to do quantitative analysis.

High Frequency Noise - The random or periodic signal fluctuation of the order of ten or more cycles per minute. This type of noise appears as a fuzzy baseline it is typically caused by the electronic of the chromatographic system.

Low Frequency Noise - This type of noise appears as very broad peaks in the chromatogram. It is most often caused by carryover of late eluting peaks from

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previous injections or low frequency electrical or electronic variations.

Matrix Noise - This is the increase in baseline noise caused by co-extractives. It may appear as a series of ill-defined and overlapping peaks on expansion of the baseline.

Peak to Peak Noise - The measured difference from the most positive noise to the most negative noise in the retention window of interest.

Primary Identification Technique and the Confirmation Technique - The least sensitive of the Identification and Confirmation Technique would be the limiting factor in detection.

Examples:

- 1a) Identification: GC NPD with a particular column.
 - 2b) Confirmation: GC MSD with an appropriate alternative column.

 - 2a) Identification: GC FPD with a particular column.
 - 2b) Confirmation: GC ELCD with an appropriate alternative column.

 - 3a) Identification: GC ELCD with a particular column.
 - 3b) Confirmation: GC ELCD with an appropriate alternative column.
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2/2/96

QA Committee Representative
1995-1996 Quality Assurance Committee
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Date

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2/1/96

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